DATA EVALUATION RECORD

HOMOBRASSINOLIDE, TECH.
OPPTS 870.3100
STUDY TYPE: SUBCHRONIC ORAL TOXICITY - RAT

MRID 47208906

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
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Primary Reviewer:
H. T. Borges, Ph.D., MT(ASCP), D.A.B.T.

Signature: Teia Borges
Date: FEB 20 2008

Secondary Reviewers:
R. A. Young, Ph.D., D.A.B.T.

Signature: Robert A. Young
Date: FEB 20 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 20 2008

Quality Assurance:
Kim Slusher, M.S.

Signature: Kimberly G. Slusher
Date: FEB 20 2008

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EPA Secondary Reviewer: _____

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DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity Gavage - Rat;
OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

PC CODE: 69361-RT **DP BARCODE:** 347313

TEST MATERIAL (PURITY): Homobrassinolide, technical (65.1% SS-isomer)

SYNONYMS: 22(S),23(S)-homobrassinolide

CITATION: Baskaran, J. (2003) Homobrassinolide, technical; 90-day oral toxicity in rats in support of FIFRA Registration. International Institute of Biotechnology and Toxicology (IIBAT), Padappai – 601 301, Kancheepuram (Dist.), Tamil Nadu, India. Laboratory report number 12285, June 29, 2003, MRID 47208906. Unpublished.

SPONSOR: Godrej Agrovet Ltd., Pirojshanagar, Eastern Express Highway, Vidhroli, Mumbai – 400 079, India

EXECUTIVE SUMMARY:

In a 91-day oral toxicity study (MRID 47208906), homobrassinolide, technical (65.1% SS-isomer, Lot No. HBR 009/02) was administered by gavage to groups of 20 male and female Wistar rats at concentrations of 0 or 1000 mg/kg bw/day. At the end of the treatment period, half the rats in each group were sacrificed while the remaining rats were sacrificed after a 28-day recovery period.

No significant treatment-related effects were noted on morbidity or mortality, body weight, food consumption, or hematological parameters following the treatment or recovery periods. No opthalmological effects were noted after the 91-day treatment period. With few exceptions, the accuracy and precision of the clinical chemistry data were unacceptable (sodium, potassium, calcium, glucose, total protein, total bilirubin, creatinine, and albumin) or inconsistent between parameters (such as BUN to creatinine; total bilirubin to AST and ALT activity; and total protein to albumin). At the end of the treatment period, the absolute and relative liver weights of treated male and female rats were statistically increased (17% and 9% in males; 51 and 38% in females, respectively), but no treatment-related effects were noted microscopically. After the recovery period, the absolute and relative liver weights of treated male and female rats were slightly different than control, but the changes were ≤7%. The absolute and relative kidney weights of female rats were significantly increased 21% and 10% after treatment, respectively, but were not significantly different from control following recovery. No treatment-related effects were noted in the kidneys microscopically. Of particular interest in this study was an ~15% increase in the absolute ovary weight of female rats after 91-days of treatment that declined to weights

consistent with control animals during the 28-day recovery period. The relative ovary weight of treated female rats was not statistically different than control rats. Since the test material is a known plant sterol, this effect is of interest relative to potential estrogenic effects.

A LOAEL or NOAEL could not be determined based on the study results. Verification of the dose and test material stability in the vehicle; the accuracy and precision of the clinical chemistry results; the potential replacement of animals included in the study; a complete list of tissues examined microscopically; discussion of potential estrogenic effects, and complete microscopic reports on all animals were not provided.

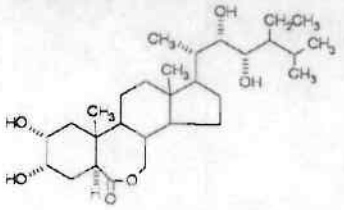
This 91-day oral toxicity study with recovery in the Wistar rat is **Unacceptable/Guideline** and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat. Specific problems with the study are outlined in the deficiency section of this MRID.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Homobrassinolide, technical

Description:	Creamish (pale yellow) powder
Lot/batch #:	HBR 009 / 02
Purity:	65.1% SS-isomer
Compound stability:	One year at room temperature; years refrigerated
CAS # of TGAI:	80483-89-2
Structure:	

2. **Vehicle and/or positive control:** Vegetable oil

3. **Test animals:**

Species:	Rat	
Strain:	Wistar	
Age/weight at study initiation:	Six – eight weeks; Males 145 – 210 g; Females 132 – 175 g	
Source:	IIBAT, Padappai – 601 301, India	
Housing:	Five animals/sex/treatment group	
Diet:	Pelleted M/s. Amrut Laboratory Animal Feed, *ad libitum*	
Water:	Filtered water, *ad libitum*	
Environmental conditions:	**Temperature:**	19.0 – 24.8°C
	Humidity:	44 - 68%
	Air changes:	Not reported
	Photoperiod:	12 hrs light/dark
Acclimation period:	One week	

B. STUDY DESIGN:

1. **In life dates:** Start: March 2003; End: June 2003 (Note: the report date on the cover of this MRID is June 29, 1993; which provides a remarkably short interval between the end of the in-life phase, review of at least 260 histopathology slides, and writing and submitting the final report.)

2. **Animal assignment:** Animals were randomly assigned (method not reported) to the test groups noted in Table 1. Rats in all groups were treated for 90 consecutive days, five days/week by gavage. At the end of the treatment period, rats in Groups 1 and 2 were sacrificed while rats in Groups 3 and 4 were sacrificed after a 28-day recovery period.

TABLE 1. Study design			
Test group	Dose (mg/kg bw/day)	# Male	# Female
1	0	10	10
2	1000	10	10
3	0	10	10
4	1000	10	10

3. **Dose selection rationale:** The dose concentrations were selected based on the results of a range finding study where groups of three male and three female rats were treated by gavage with 100, 500, or 1000 mg/kg bw/day test material for 14 days. No treatment-related effects were found.

4. **Dose preparation:** The doses were prepared by mixing the appropriate amount of test material in vegetable oil. Dose volume for all groups was 10 mL/kg bw. The frequency of dose preparation, whether the doses were adjusted for the concentration of active ingredient, and what composed the remaining 35% of the neat test material were not reported.

Results:

Homogeneity analysis: Homogeneity of the dosing solutions was not reported.

Stability analysis: Stability analysis of the dosing solutions was not reported.

Concentration analysis: Concentration analyses were not reported.

5. **Statistics:** Statistical comparisons of each treatment group with its respective control group were done by the Student's t-test at 5% significance.

C. **METHODS:**

1. **Observations:**

1a. **Cageside observations:** Animals were inspected daily for signs of toxicity and mortality.

1b. **Clinical examinations:** Clinical examinations general health, the autonomic and central nervous system activity, somatomotor activity and behavioral abnormalities were conducted daily.

1c. **Neurological evaluations:** Detailed neurological examinations were not done.

2. **Body weight:** Animals were weighed weekly.

3. **Food consumption and compound intake:** Food consumption of each cage of five animals/ sex/group was determined weekly and reported as g/animal/week. Food efficiency was not reported. Compound intake is in Table 1.

4. **Ophthalmoscopic examination:** The eyes of all rats in Groups 1 and 2 were examined

before the start and at the end of treatment. The eyes were examined with the aid of an ophthalmoscope. Further details were not provided.

5. **Hematology and clinical chemistry:** Blood was collected from all animals in Groups 1 through 4 on Day 91 and from all animals in Groups 3 and 4 at the end of the recovery period for hematology and clinical chemistry. The length of fasting prior to blood collection was not reported. Blood was collected into EDTA for hematology analyses and heparin for clinical chemistry. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count (WBC)*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
X	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus		Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg. *)	X	Total bilirubin
	Alkaline phosphatase (ALK)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase (CPK)		
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase* (SDH)		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase (GDH)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis:** Urinalysis was not done.

7. **Sacrifice and pathology:** All animals that died and those sacrificed on schedule were subjected to gross pathological examination. Rats in Groups 1 and 2 were sacrificed on Day 91 of the study while rats in Groups 3 and 4 were sacrificed on Day 119. A complete list of collected tissues was not provided, but data on those tissues marked with an "X" indicates

that at least these were examined microscopically. The tissues were collected and preserved in 10% buffered formalin, embedded in paraffin, sectioned at 3-5 µm, and stained with hematoxylin and eosin prior to examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta*		Brain*+
	Salivary glands*	X	Heart*+		Peripheral nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)*
	Stomach*		Lymph nodes*		Pituitary*
X	Duodenum*	X	Spleen*+		Eyes (optic nerve)*
X	Jejunum*		Thymus*+	X	GLANDULAR
X	Ileum*				Adrenal gland*+
	Cecum*	X	UROGENITAL		Lacrimal gland
	Colon*	XX	Kidneys*+		Parathyroid*
	Rectum*	X	Urinary bladder*		Thyroid*
XX	Liver*+	XX	Testes*+	X	OTHER
	Gall bladder (not rat)*		Epididymides*+		Bone (sternum and/or femur)
	Bile duct (rat)		Prostate*		Skeletal muscle
	Pancreas*		Seminal vesicles*		Skin*
X	RESPIRATORY	XX	Ovaries*+		All gross lesions and masses*
	Trachea*	X	Uterus*+		
X	Lung*		Mammary gland*		
	Nose*				
	Pharynx*				
	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No clinical signs of toxicity were observed. Review of the clinical toxicity observation data suggests that five males and two females in Group 1, one male in Group 2, and one male in Group 3 were replaced between days 21 and 22. No explanation was provided. It was also not reported whether the replacement animals had been treated the preceding 21 days.

2. **Mortality:** All rats survived until scheduled sacrifice.

B. BODY WEIGHT AND WEIGHT GAIN:

Although there was a slight increase in body weight gain by Group 2 male and female rats, no toxicologically significant effects were noted on body weight or body weight gain (Table 2).

TABLE 2. Average body weights and body weight gains during 90 days of treatment							
Group	Dose (mg/kg bw/day)	Body weight (g ± SD)				Total Weight Gain	
		Week 1	Week 7	Week 13	Week 17	g	% difference from control
Males							
1	0	188.6 ± 19.59	274.4 ± 25.23	305.8 ± 27.77	-	117.2	-
2	1000	179.6 ± 15.95	287.3 ± 26.24	327.7 ± 30.07	-	148.1	26.3
3	0	182.4 ± 12.48	293.4 ± 29.74	345.4 ± 36.99	368.6 ± 34.46	186.2	-
4	1000	188.0 ± 24.58	281.6 ± 26.45	330.7 ± 37.11	354.0 ± 39.02	166.0	-11
Females							
1	0	154.3 ± 8.43	199.8 ± 20.21	195.6 ± 23.06	-	41.3	-
2	1000	154.8 ± 14.50	199.1 ± 13.97	213.1 ± 9.77	-	58.3	41
3	0	157.2 ± 9.47	206.5 ± 20.32	218.4 ± 25.26	232.7 ± 27.44	75.5	-
4	1000	154.4 ± 10.55	206.1 ± 11.15	215.6 ± 14.11	230.7 ± 20.62	76.3	1

Data from pages 25-26 of MRID 47208906
N=10 for all groups

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Although sporadic statistically significant increases and decreases between treated and control rats were observed, overall food consumption was not affected by treatment.

2. **Compound consumption:** Compound consumption is shown in Table 1 above.

D. OPHTHALMOSCOPIC EXAMINATION:

No treatment-related effects were observed.

E. BLOOD ANALYSES:

1. **Hematology:** Although statistically significant decreases in the HCT of Group 2 male rats and increases in the WBC count and coagulation times of Group 4 male rats were found at 91 days, all were slight variations and within the normal range for the parameter established by the performing laboratory. No statistically significant treatment-related effects were found in female rats. Slight variations in the coagulation times of male and female rats following the 28-day recovery were not of toxicological relevance.

2. **Clinical chemistry:** Review of the clinical chemistry data shows that much, if not all of the data, is unreliable at both sampling intervals. For instance, the standard deviation of the sodium data is totally unacceptable for interpretation; being approximately an order of magnitude higher than what is acceptable. Following review of the individual animal data, most sodium results, whether increased or decreased, were incompatible with life, while the average is typically at the upper end of the normal range for animals of this species. Likewise, similar conclusions can be made for the potassium and calcium data. While the enzymatic activities of AST and ALT appear acceptable, the bilirubin concentrations for rats of the strain were quite variable and often increased; something not to be expected from "normal" animal data for these parameters. A similar effect is seen where the creatinine concentration for each group does not correspond with the reported BUN. While the average

glucose concentration for each group appears acceptable, the standard deviation is dramatically increased; indicative of wide variation in the individual animal results. For most all clinical chemistry analyses, the individual animal variation dramatically exceeds that expected. Based on these assessments, the reviewer considers the clinical chemistry data unacceptable and not conducive to interpretation.

F. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** As shown in Table 3, both the absolute and relative liver weights of treated male and female rats were statistically increased following 91 days of treatment. After the 28-day recovery period, the liver weight decreased and was only slightly different than the rats respective controls. The absolute left and right kidney weight of treated female rats was statistically increased 21% and 10%, respectively, following 91 days of treatment; however, the increase was ≤8% following the recovery period. The significance of this affect is unknown. The absolute left and right ovary weight of treated female rats was increased ~15% after the 91-day treatment period, but was not increased following the recovery period. This suggests that the increased weight is related to treatment, but it cannot be substantiated with the microscopic data and may be a spurious event.

TABLE 3. Absolute and relative organ weight changes in rats treated with homobrassinolide[1]

Organ	Group 1	Group 2	Group 3	Group 4
		Males		
Body Wt. (g)	305.8 ± 8.78	327.7* ± 9.51 (7)	368.6 ± 10.90	354.0* ± 12.34 (-4)
Liver				
Absolute (g)	8.683 ± 0.318	10.154* ± 0.347 (17)	11.444 ± 0.645	11.642 ± 0.586
Relative (%)	2.851 ± 0.105	3.104* ± 0.084 (9)	3.098 ± 0.129	3.278* ± 0.082 (6)
Kidney (Left)				
Absolute (g)	1.108 ± 0.040	1.161* ± 0.034 (5)	1.188 ± 0.057	1.051* ± 0.031 (-7)
Relative (%)	0.365 ± 0.016	0.357* ± 0.013 (-2)	0.324 ± 0.016	0.301* ± 0.015 (-6)
Kidney (Right)				
Absolute (g)	1.090 ± 0.034	1.130* ± 0.024 (4)	1.200 ± 0.051	1.125* ± 0.049 (-6)
Relative (%)	0.359 ± 0.014	0.348* ± 0.013 (3)	0.328 ± 0.016	0.322 ± 0.020
Testis (Left)				
Absolute (g)	1.474 ± 0.017	1.600* ± 0.040 (8)	1.509 ± 0.024	1.524 ± 0.025
Relative (%)	0.485 ± 0.013	0.491 ± 0.015	0.413 ± 0.014	0.434* ± 0.013 (5)
Testis (Right)				
Absolute (g)	1.476 ± 0.023	1.587* ± 0.046 (8)	1.531 ± 0.021	1.518 ± 0.023
Relative (%)	0.485 ± 0.013	0.487 ± 0.016	0.419 ± 0.014	0.433* ± 0.014 (3)

TABLE 3. Absolute and relative organ weight changes in rats treated with homobrassinolide[1] (cont.)				
Organ	Group 1	Group 2	Group 3	Group 4
		Females		
Body Wt. (g)	195.6 ± 7.29	213.1* ± 3.09 (9)	232.7 ± 8.68	230.7 ± 6.52
Liver				
Absolute (g)	5.398 ± 0.124	8.172* ± 0.248 (51)	6.509 ± 0.246	6.126* ± 0.209 (-6)
Relative (%)	2.786 ± 0.094	3.844* ± 0.138 (38)	2.867 ± 0.219	2.664* ± 0.084 (-8)
Kidney (Left)				
Absolute (g)	0.666 ± 0.022	0.805* ± 0.019 (21)	0.806 ± 0.025	0.871* ± 0.023 (8)
Relative (%)	0.345 ± 0.016	0.379* ± 0.013 (10)	0.353 ± 0.021	0.380* ± 0.013 (8)
Kidney (Right)				
Absolute (g)	0.675 ± 0.019	0.817* ± 0.016 (21)	0.829 ± 0.022	0.811 ± 0.027
Relative (%)	0.349 ± 0.016	0.385* ± 0.012 (10)	0.360 ± 0.012	0.355 ± 0.018
Ovary (Left)				
Absolute (g)	0.074 ± 0.003	0.085* ± 0.004 (15)	0.077 ± 0.004	0.076 ± 0.004
Relative (%)	0.039 ± 0.002	0.040 ± 0.002	0.034 ± 0.002	0.033 ± 0.002
Ovary (Right)				
Absolute (g)	0.072 ± 0.003	0.082* ± 0.004 (14)	0.083 ± 0.004	0.080 ± 0.004
Relative (%)	0.037 ± 0.002	0.039 ± 0.002	0.036 ± 0.002	0.035 ± 0.002

Data from pages 71-74 of MRID 47208906
[1]Statistical analyses (Student's t-test) repeated by reviewer since validation of report analyses could not be confirmed
* $p \leq 0.05$
Results in parentheses are percent difference relative to respective control calculated by reviewer.
N=10 for all groups

2. **Gross pathology:** No treatment-related effects were noted.

3. **Microscopic pathology:** No treatment-related effects were noted. Changes in absolute and/or relative liver, kidney, or ovary weights were not correlated with microscopic data. (Limited individual animal data were presented.)

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The study author concluded that the test material did not induce clinical signs of toxicity, alter body weight or food consumption, affect mortality, hematology or clinical chemistry parameters, organ weight, or macro- or micropathology in rats following a 91-day treatment period with 1000 mg/kg bw/day. The study author established a NOAEL of 1000 mg/kg bw/day.

B. REVIEWER COMMENTS:

In this study, groups of 20 male and 20 female Wistar rats were treated by gavage with 0 or 1000 mg/kg bw/day test material five days/week for 91 days. At the end of the treatment period, half the rats in each group were sacrificed while the remaining rats were sacrificed following a 28-day recovery period.

No significant treatment-related effects were noted on morbidity or mortality, body weight, food consumption or hematological parameters following the treatment or recovery periods.

No opthalmological effects were noted. Because of extreme variation, clinical chemistry data were unsuitable for interpretation after either the treatment or recovery period. At the end of the treatment period, the absolute and relative liver weights were statistically increased in treated male and female rats (17% and 9% in males; 51 and 38% in females, respectively), consistent with hypertrophy, but no treatment-related effects were noted microscopically. After the recovery period, the absolute and relative liver weights of treated male and female rats were slightly different than control, but the changes were ≤7% and not of biological or toxicological interest. The absolute and relative kidney weights of female rats were significantly increased 21% and 10% after treatment, respectively, but were not significantly different from control following the recovery period. Again, no relevant treatment-related effect was noted in the kidneys microscopically. Of particular interest in this study was an ~15% increase in the absolute ovary weight of female rats after 91-days of treatment that declined during the 28-day recovery period. The relative ovary weight of treated female rats was not statistically different than control rats at any time during the study. Since the test material is a known plant sterol, this particular effect is of interest relative to potential estrogenic effects and was not addressed by the study authors.

Based on the potential estrogenic effect of the test material and the study deficiencies noted below, a LOAEL or NOAEL could not be identified.

C. STUDY DEFICIENCIES:

A number of major deficiencies in the study report were noted. These include:

Quantitative measurements of the dosing solutions, number of dose preparations, prepared dose stability, prepared dose homogeneity, and whether the dose was adjusted to the concentration of the active ingredient were not reported. This could be resolved with submission of the appropriate data.

A complete list of tissues collected and examined microscopically following necropsy was not provided. This could be resolved with submission of the appropriate data. However, the only organs weighed were the liver, kidney, and testes/ovaries. No other organ weights were measured.

With few exceptions, the accuracy and precision of the clinical chemistry data was unacceptable (sodium, potassium, calcium, glucose, total protein, total bilirubin, creatinine, and albumin). Much of the individual animal sodium, potassium, and calcium results are incompatible with life. Additionally, explanations of why several other parameters were not consistent with each other (such as BUN to creatinine; total bilirubin to AST and ALT activity; and total protein to albumin) were not presented. It is unlikely that an acceptable explanation can be provided.

An explanation of why individual animal numbers differed for several rats of Groups 1, 2, and 3 from day 1 to day 22 of the study was not provided. It was not reported that if the animals were replaced, whether they were treated for the preceding 21 days.

Statistical analyses presented in the study report could not be verified by repeat analysis.